Claims

[1] A MδLK8 recombinant expression vector containing LK8 expression cassette comprising promoter, secretion sequence, LK8 cDNA represented by SEQ ID No: 1 and terminator in that order, δ sequence for the multiple insertion of LK8 expression cassette into chromosome of a host strain, and neomycin resistant gene (neo) for the selection after the multiple insertion.

[2] The MδLK8 recombinant expression vector according to claim 1, wherein said promoter is GAL1 promoter, secretion sequence is α-factor secretion signal represented by SEQ ID No: 2, and terminator is CYC1 terminator.

[3] A transformed *Saccharomyces cerevisiae* strain prepared by transfecting a host strain with the vector of claim 1.

[4] The transformed *Saccharomyces cerevisiae* strain according to claim 3, wherein said host strain is selected from a group consisting of *Saccharomyces cerevisiae* BJ3501, *Saccharomyces cerevisiae* BY4742, *Saccharomyces cerevisiae* CEN.PK2-1D and *Saccharomyces cerevisiae* 2805.

[5] The transformed *Saccharomyces cerevisiae* strain according to claim 3, wherein said strain is *Saccharomyces cerevisiae* BJ3501/MδLK8 #36 (Accession No: KCTC 10582BP).

[6] A method for preparing a transformant expressing LK8 protein highly, comprising the following steps:
(1) Transforming a host strain with the recombinant vector of claim 1;
(2) Culturing the transformant prepared in the step 1 after the treatment of G418 sulfate antibiotics; and
(3) Selecting LK8 high expressing transformant by immunoassay.

[7] The method according to claim 6, wherein said G418 is treated by 5 - 20 g/L.

[8] The method according to claim 6, wherein said immunoassay is selected from a group consisting of colony immunoblotting assay, dot blotting assay and ELISA (enzyme linked immunosorbant assay).

[9] The method according to claim 6, wherein said step 3 is repeated once to three times.

[10] The method according to claim 6, wherein said step 3 consists of the following steps: (a) primary selection by colony immunoblotting; (b) secondary selection by dot blotting from the primary selected strains; and (c) final selection by ELISA from the secondly selected strains.

[11] A method for mass-production of LK8 protein comprising the following steps:
(1) Preparing a transformed strain by inserting the recombinant LK8 gene expression vector of claim 1 into a host strain;

(2) Seed-culturing the transformed strain prepared in the step 1 and batch-culturing the strain in a liquid medium containing glucose and galactose as a carbon source, with keeping dissolved oxygen stable by regulating air supply and/or stirring speed;

(3) Fed-batch-culturing the culture solution of the step 2 with a feed medium containing galactose; and

(4) Purifying LK8 protein from the culture solution of the step 3.

[12] The method according to claim 11, wherein said transformed strain of step 1 is a transformed *Saccharomyces cerevisiae* strain of claim 3.

[13] The method according to claim 11, wherein said batch-culture of step 2 is performed with 1 - 3 vvm (5 - 80 L/minute) of air supply and/or 200 - 1000 rpm of stirring speed, in a liquid medium containing 1 - 5%(w/v) glucose and 1 - 5%(w/v) galactose as a carbon source, in which dissolved oxygen is adjusted to 40 - 90% of maximum dissolved oxygen.

[14] The method according to claim 11, wherein said fed-batch-culture of step 3 is performed using a liquid medium containing 10 - 50%(w/v) of galactose as a carbon source and regulating the supply speed of the feed medium in order to maintain the content of galactose in the medium as 0.5 - 5%(w/v).

[15] The method according to claim 11, wherein said purification of LK8 protein of step 4 is performed by chromatography.

[16] The method according to claim 15, wherein said chromatography includes ion exchange chromatography and hydrophobic interaction chromatography.

[17] The method according to claim 16, wherein said exchange chromatography is cation exchange chromatography and the elution of LK8 protein is performed with an eluting buffer (pH 4.0 - 8.0) containing 0 - 5 M NaCl.

[18] The method according to claim 16, wherein said hydrophobic interaction chromatography is performed with 0 - 100 mM sodium phosphate eluting buffer (pH 4 - 8) containing 0.1 - 5 M ammonium sulfate and 0 - 500 mM NaCl for the elution of LK8 protein.